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Efficacy of a modified live *Edwardsiella ictaluri* vaccine in channel catfish as young as seven days post hatch

Craig A. Shoemaker [a,*], Phillip H. Klesius [a], Joseph M. Bricker [b]

[a] *Fish Diseases and Parasites Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Box 952, Auburn, AL 36830, USA*
[b] *Intervet, 405 State Street, Millsboro, DE 19966, USA*

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Abstract

Channel catfish (*Ictalurus punctatus*) were vaccinated by immersion at 12, 14, 16 and 31 days post hatch with modified live *Edwardsiella ictaluri* RE-33 vaccine in 1997 and 7 and 10 days post hatch in 1998. At 20 to 21 days post vaccination, the groups of vaccinates and non-vaccinates were challenged with virulent *E. ictaluri* and monitored for mortality for at least 14 days following challenge. Results showed the vaccine to be efficacious in channel catfish as young as 7 days post hatch with relative percent survival ranging from 58.4 to 77.5.

Keywords: Modified live vaccine; *Edwardsiella ictaluri*; Channel catfish; Days post hatch

1. Introduction

Enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri*, is responsible for more than US$20 to 30 million losses annually to the channel catfish industry in the United States (Plumb and Vinitnantharat, 1993). An effective vaccine which can be delivered to catfish prior to release in production ponds (about 10 days post hatch [DPH]) is desirable. Attempts to vaccinate young catfish with killed vaccines have failed. Thune et al. (1997) attempted to vaccinate channel catfish (*Ictalurus punctatus*)

* Corresponding author. Tel.: +1-334-887-3741; Fax: +1-334-887-2983; E-mail: cshoemak@acesag.auburn.edu

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12 DPH with a formalin killed *E. ictaluri* vaccine by immersion or immersion followed by an oral booster. No protection resulted from either vaccination scheme. Recently, Klesius and Shoemaker (1999) produced an effective modified live *E. ictaluri* vaccine which could be administered by immersion to successfully vaccinate catfish 3–9 months of age. The objective of this study was to determine if the modified live *E. ictaluri* vaccine would be efficacious in young (7 DPH) channel catfish.

2. Materials and methods

2.1. Experimental animals

Channel catfish eggs were hatched at the USDA, ARS Fish Diseases and Parasites Research Laboratory in Auburn, AL. Prior to experimentation, 50 channel catfish fry were homogenized and cultured by the enrichment technique of Klesius (1992). No *E. ictaluri* was isolated from any of the fish. Fish were stocked into 55-l aquaria supplied with flow-through water at a rate of 0.5 l/min. In 1997 (Table 1), fish (48 to 100 per group) were vaccinated by immersion for 2 and 10 min prior to being divided into equal numbers in triplicate aquarium where they were held until challenge. Trials in 1998 consisted of 125 vaccinated fish (2-min immersion exposure) and 125 control fish per

Table 1
Summary of modified live *E. ictaluri* RE-33 vaccination of channel catfish at 12, 14, 16 and 31 days post hatch (1997)

Days post hatch vaccinated[1]	Duration (min) of vaccination[2]	Percent mortality[3] (SEM)[4]	Relative percent survival (RPS)[5]
12	2	33.3 ($\pm$5.48)[a]	57.6
	10	26.8 ($\pm$3.85)[a]	65.3
14	2	22.5 ($\pm$3.55)[a]	70.9
	10	25.3 ($\pm$4.15)[a]	67.9
16	2	16.1 ($\pm$1.46)[a]	79.5
	10	42.2 ($\pm$11.93)[a]	45.3
31	2	21.3 ($\pm$4.97)[a]	73.0
	10	28.4 ($\pm$0.62)[a]	63.9
Non-vaccinates	–	78.7 ($\pm$8.73)[b]	–

[1]Vaccine prepared from frozen stock at the USDA, ARS laboratory.
[2]Fish (48 to 100 per time and duration) were immersion vaccinated in 5×10^5 to 1×10^6 CFU/ml *E. ictaluri* RE-33 for 2 or 10 min.
[3]Fish were challenged when all were at least 50 days post hatch or at least 20 days after vaccination. Fish were challenged by immersion for 30 min in 7.5 l of water containing 1×10^7 CFU/ml *E. ictaluri* AL-93-75 in triplicate. After challenge, fish were returned to their respective aquariums. The control group (75 fish) was three tanks of 25 fish per aquarium.
[4]Data were analyzed by the general linear models procedure of SAS (SAS Institute, 1997) with Duncan's multiple range test to compare means. Standard error of the means are presented in parentheses. Means with different letters are significantly different ($P < 0.05$).
[5]Relative percent survival was calculated according to the method of Amend (1981).

age and vaccine preparation (Table 2). Daily water temperature was 25 ± 1°C and dissolved oxygen ranged between 6 and 8 mg/l. A 12:12 h light:dark schedule was maintained. The fish were fed with commercial catfish fry feed.

2.2. Vaccine preparation and administration

In 1997, the vaccine was prepared according to Klesius and Shoemaker (1999). Briefly, one vial of *E. ictaluri* RE-33 passaged two times from the Master Seed (MS) was used to inoculate brain heart infusion broth. The RE-33 MS X + 3 broth culture (250 ml) was incubated in a water bath at 25–27°C with shaking at 50 rpm for 24 h before vaccination. In 1998, freeze dried vaccines produced by Intervet (Intervet, 405 State Street, Millsboro, DE 19966, USA) were used. The vaccines were designated as Immuno X + 5 (original RE-33 passaged five times at Intervet prior to freeze drying), Immuno2 X + 5 (original RE-33 passaged five times in media without animal by-products at Intervet prior to freeze drying) and Serial 1 A (first production batch for field trials). The RE-33 MS X + 3 was prepared as in 1997 for comparison to the freeze dried product. The modified live *E. ictaluri* vaccine was administered to catfish which ranged in age from 12,14, 16 and 31 DPH by immersion for 2 min or 10 min (Table 1) and 7 and 10 DPH by immersion for 2 min (Table 2). The dose utilized for the studies is

Table 2
Efficacy of modified live *E. ictaluri* RE-33 vaccination[1] of channel catfish at 7 and 10 days post hatch (1998)

Days post hatch vaccinated	Vaccine preparation designation or non-vaccinates[2]	Vaccination dose[3]	Number of fish	Percent mortality[4]	Relative percent survival (RPS)[5]
7	Immuno X+5	7.0×10^5	125	8.1	77.5
7	Non-vaccinates	None	125	36.0	–
7	Serial 1 A	5.2×10^5	125	12.8	58.4
7	Non-vaccinates	None	125	30.4	–
7	Immuno2 X+5	1.0×10^6	125	10.4	66.2
7	Non-vaccinates	None	125	30.4	–
10	Immuno2 X+5	1.6×10^6	125	6.5	78.9
10	Non-vaccinates	None	125	30.4	–
10	Serial 1 A	5×10^5	250	12.8	71.9
10	Non-vaccinates	None	250	45.6	–
10	RE-33 MS X+3	9.8×10^5	125	11.2	64.1
10	Non-vaccinates	None	125	31.2	–

[1]Fish were vaccinated in 100 ml immersion water and vaccine for 2 min immersion exposure before being returned to aquarium.
[2]Vaccine was prepared by Intervet (lyophilized product); except for the RE-33 MS X+3 which was prepared from frozen stock at the USDA-ARS laboratory.
[3]Vaccination dose was determined from five replicate plate counts from immersion water used to vaccinate fish.
[4]Fish were challenged 21 days after vaccination and mortality was monitored for a period of 14 days after challenge. Fish were challenged by immersion for 30 min in 7.5 l of water containing 1×10^7 CFU/ml *E. ictaluri* AL-93-75.
[5]Relative percent survival was calculated according to Amend (1981).

shown in Table 1 (footnote number 2) and Table 2 as determined by five replicate plate counts from the tank water. Dead vs. alive bacteria in the freeze dried preparations was not determined, however, a proprietary vaccine stabilizer was used to minimize bacterial cell death in the freeze drying process.

2.3. Experimental challenge

All fish (vaccinates and non-vaccinates) were challenged by immersion exposure to virulent *E. ictaluri* (isolate AL-93-75) at a concentration of 1×10^7 CFU/ml (Klesius and Shoemaker, 1997) for 30 min prior to being released back into the respective aquaria. Mortalities were monitored twice daily for 14 days after challenge. No ESC deaths were noted to occur 10 days after challenge (Klesius and Shoemaker, 1997). Clinical signs of enteric septicemia of catfish, which include petechial hemorrhage in the skin under jaw and on the operculum and abdomen often becoming bright red (paintbrush hemorrhage), hemorrhage at base of fins and abdominal distention, were observed in mortalities (Plumb, 1994). Necropsies were performed and anterior kidney tissue from dead fish was cultured to confirm death due to *E. ictaluri* (Klesius, 1992). The efficacy of the vaccine in 7 to 31 DPH catfish was calculated as relative percent survival (RPS) (Amend, 1981).

3. Results

Efficacy of modified live *E. ictaluri* vaccination in channel catfish 7, 10, 12, 14, 16 and 31 DPH is shown in Tables 1 and 2. In 1997, RPS values ranged from 45.3 to 79.5%. Protection was seen in 12, 14, 16 and 31 days post hatch channel catfish vaccinated for 2 min in 1997. Marginal protection was observed in the group of 16 DPH fish vaccinated for 10 min. However, this protection did not meet the criteria as defined by Amend (1981). In 1998, RPS values in 7 DPH fish ranged from 58.4 to 77.5 using three different vaccine preparations and a 2-min immersion exposure. Protection was also seen in 10 DPH catfish vaccinated for 2 min in 1998 with RPS's of 64.1 to 78.9 dependent on vaccine preparation.

4. Discussion

The results of the present study show that channel catfish which are 7 DPH can be successfully vaccinated by 2-min immersion using the modified live *Edwardsiella ictaluri* vaccine. We observed marginal protection in the 10 min immersion vaccination, but protection was observed in the 2 min immersion vaccination of 16 DPH channel catfish. The reason for this is unknown. We did not observe any relationship between increased age of the vaccinates or exposure times and the degree of protection.

Protective immunity to *E. ictaluri* is dependent on both the cellular immune response to (Antonio and Hedrick, 1994; Shoemaker and Klesius, 1997; Shoemaker et al., 1997) and challenge isolate of *E. ictaluri* (Klesius and Shoemaker, 1997). Klesius and

Shoemaker (1999) demonstrated that the modified live *E. ictaluri* vaccine stimulated strong acquired immunity in 3–9 month old channel catfish against a number of *E. ictaluri* isolates. Fish vaccinated with the modified live *E. ictaluri* vaccine in that study were protected for at least 4 months. The modified live *E. ictaluri* vaccine will satisfy the needs of the catfish industry because it can be administered by immersion exposure to young channel catfish.

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